

WE CLAIM

1. A process for the preparation of an agglutination reagent for rapid and early detection of typhoid, comprising in the steps of :
 - (a) preparing *Salmonella typhi* specific antibody
 - 5 (b) preparing latex particles suspension
 - (c) coating said latex particles with the antibody
2. A process as claimed in claim 1 wherein Flagellin gene sequence specific to *Salmonella typhi* is cloned and expressed by recombinant DNA technology.
- 10 3. A process as claimed in claim 2 wherein the expressed recombinant protein is purified by affinity chromatography.
4. A process as claimed in claim 3 wherein the hyper immune sera against the recombinant protein is raised in rabbit.
5. A process as claimed in claim 4 wherein the immunoglobulin fraction of hyper immune sera is separated by ammonium sulphate precipitation.
- 15 6. A process as claimed in claim 1 wherein the precipitated immunoglobulins are suspended in phosphate buffer dialysed and the protein content is determined.
- 20 7. A process for the preparation of an agglutination reagent for rapid and early detection of typhoid as claimed in claim 1 wherein 1% carboxylated latex particles of size 0.88 to 0.90 μm and 40mM 2-N Morpholinoethane sulphonic acid (MES) buffer of pH 5.5 to 6.0 are taken in a preferred ratio of 1:1.
- 25 8. A process for the preparation of an agglutination reagent for rapid and early detection of typhoid as claimed in claim 1 wherein the suspension of latex particles in MES buffer is mixed on a vortex mixer for around 60 seconds and centrifuged at 10,000 rpm for 10-12 minutes at about 4°C.

9. A process for the preparation of an agglutination reagent for rapid and early detection of typhoid as claimed in claim 1 wherein the latex particles are further washed twice with 20mM MES buffer of pH 5.5 at 10,000 rpm for 10-12 minutes at about 4°C.
- 5 10. A process for the preparation of an agglutination reagent for rapid and early detection of typhoid as claimed in claim 1 wherein the washed latex particles are suspended in 20mM MES buffer of pH 5.5 and the volume is made up as equal to the starting volume of latex particles.
- 10 11. A process for the preparation of an agglutination reagent for rapid and early detection of typhoid as claimed in claim 1 wherein the suspended latex particles are sonicated by a tip sonicator at about 5 watts for 60-120 seconds preferably 90 seconds.
- 15 12. A process for the preparation of an agglutination reagent for rapid and early detection of typhoid as claimed in claim 1 wherein freshly prepared solution of 0.1 M 1-ethyl-3 (3-dimethyl-amino propyl) carbodiimide hydrochloride (EDC) in 20mM MES buffer of pH 5.5, in a preferred ratio of 1:1 is added drop wise to the latex particles suspension, while the suspension is slowly vortexed.
- 20 13. A process for the preparation of an agglutination reagent for rapid and early detection of typhoid as claimed in claim 1 wherein the latex particles with EDC solution are rotated slowly end-over-end for about 3 hours at a temperature of 20-25°C and washed thrice with 20mM MES buffer (pH 5.5) at 10,000 rpm for 10-12 minutes at a temperature of about 4°C.
- 25 14. A process for the preparation of an agglutination reagent for rapid and early detection of typhoid as claimed in claim 1 wherein the latex particles are resuspended in MES buffer (20mM, pH5.5) and sonicated by a tip sonicator for 60-120 seconds at about 5 watts.

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15. A process for the preparation of an agglutination reagent for rapid and early detection of typhoid as claimed in claim 1 wherein to the latex particle suspension prepared in step (b), 0.6-1.0 mg preferably 0.8 mg per ml of the immunoglobulins prepared in step (a) are added.
16. A process for the preparation of an agglutination reagent for rapid and early detection of typhoid as claimed in claim 1 wherein the suspension of latex particles and immunoglobulins is rotated end-over-end for 18-20 hours at a temperature of about 20-25°C.
17. A process for the preparation of an agglutination reagent for rapid and early detection of typhoid as claimed in claim 1 wherein the coating reaction is stopped by 1M glycine (pH 11.0) taken in quantity of 0.06 ml per ml of solution of immunoglobulin coated latex particles.
18. A process for the preparation of an agglutination reagent for rapid and early detection of typhoid as claimed in claim 1 wherein the coated latex particles are pelleted out by centrifugation at 10,000 rpm for 10-12 minutes at a temperature of about 4°C.
19. A process for the preparation of an agglutination reagent for rapid and early detection of typhoid as claimed in claim 1 wherein the pellet of coated latex particles is washed thrice with washing buffer comprising of 50mM glycine, pH8.5; 0.03% triton X-100 and 0.05% sodium azide; at 10,000 rpm for 10-12 minutes at a temperature of about 4°C.
20. A process for the preparation of an agglutination reagent for rapid and early detection of typhoid as claimed in claim 1 wherein the washed and coated latex particles are suspended in storage buffer comprising of 50mM glycine pH8.5; 1.0% bovine serum albumin; 0.03% triton X-100; 0.1% sodium azide and 0.01% thiomersal, to a final concentration of 1%.

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21. A process for the preparation of an agglutination reagent for rapid and early detection of typhoid as claimed in claim 1 wherein the 1% suspension of coated latex particles is sonicated by a tip sonicator for around 60 seconds at about 5 watts and stored at 4°C.
- 5 22. A process for the preparation of an agglutination reagent as substantially described and illustrated herein.